INTERRELATIONSHIPS AMONG TEMPERATURE, METABOLISM, SWIMMING PERFORMANCE AND RECOVERY IN PACIFIC COD (*Gadus macrocephalus*): IMPLICATIONS OF A CHANGING CLIMATE

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A

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Abstract

Physiological constraints are suggested to contribute to the observed changes in relative abundance of Pacific cod (*Gadus macrocephalus*) seen in association with interdecadal changes in sea surface temperatures. To examine this concept, two experiments were conducted to determine critical swimming speed (U_{crit}), rates of oxygen consumption and recovery post-exhaustion of adult cod acclimated to different temperatures. In addition, hematocrit and plasma concentrations of cortisol, metabolites and ions from resting and exhausted fish were measured to assess the impact of swim trials on fish condition. In experiment one, fish acclimated to 4°C had similar mean U_{crit} (1.07 BL/s) and resting metabolic rates (35.34 mg $O_2/kg^{0.8}/hr$) compared to fish acclimated to 11°C fish (1.07 BL/s; 49.43 mg $O_2/kg^{0.8}/hr$). Similarly, concentrations of blood constituents differed little between temperature treatments; each exhibited increases in plasma cortisol and metabolites from pre- to post-swim. Experiment two illustrated few differences in rates of recovery between temperature groups (2 and 7°C). After four hours of recovery there was no evidence of plasma cortisol or metabolites returning to pre-swim concentrations in either temperature group. It seems unlikely that physiological constraints on the metabolic performance of adult Pacific cod contribute to changes in their relative abundance.

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Introduction

The mean surface temperature of the North Pacific Ocean is known to fluctuate on an interdecadal scale alternating between warm and cold regimes (Mantua *et al.*, 1997; Lynn and Bograd, 2002; Peterson and Schwing, 2003). These fluctuations tend to be slight, normally less than 1°C (Hare and Mantua, 2000); however, small shifts in temperature have been associated with large impacts on abundance and distribution of marine organisms. In the late 1970's, following 30 years of relatively cold sea-surface-temperatures, the climate of the North Pacific shifted to a warm regime lasting through the late 1990's (Francis and Hare, 1994; Mantua *et al.*, 1997). During this regime, the abundance of forage species such as pandalid shrimp (*Pandalus sp.*) and capelin (*Mallotus villosus*) decreased (Anderson and Piatt, 1999) while recruitment of groundfish such as Pacific cod (*Gadus macrocephalus*) and pollock (*Theragra chalcogramma*) increased (Hollowed and Wooster, 1992). Since 1998, however, sea-surface temperature in the North Pacific has tended to decrease and there are indications of a shift from a warm to a cold regime (Schwing and Moore, 2000). Already, there are biological manifestations of a shift including increased biomass of forage species and decreased biomass of groundfish and salmon (Peterson and Schwing, 2003).

Shifts in ocean climate have been a major factor in the success and failure of fisheries in the Pacific. The most well described impacts were the collapse of the California sardine fishery in the 1940's and the establishment of the Peruvian anchovy

fishery from the 1950's to the 1970's, the largest individual species fishery at the time. These changes were coincident with a climate shift from a warm regime that lasted from the 1920's until the 1950's to a cold regime that lasted from the 1950's to the 1970's (Chavez *et al.*, 2003). A similar situation is noted in the Pacific salmon fishery over the past century. During the 1960's and early 1970's, harvest levels of the Alaskan salmon fisheries were low while harvest levels in the Pacific Northwest were high as compared to historical averages. However, this trend was reversed from the late 1970's to the 1990's (Mantua *et al.*, 1997). Similar catch fluctuations with temperature changes have also been observed in the groundfish fishery in Alaska with catches peaking in the 1960's and in the 1980's (Mueter, 2004).

The groundfish fishery in Alaska is the largest of the Alaskan commercial fisheries in terms of value. Groundfish include pollock, Pacific cod, sablefish (*Anoplopoma fimbria*), Atka mackerel (*Pleurogrammus monopterygius*), lingcod (*Ophiodon elongates*) and a number of rockfish and flatfish species. In 2004, the total groundfish catch in Alaska was 2.2 million metric tons with an ex-vessel value of $593 million (Hiatt *et al.*, 2005). Groundfish accounted for 51% of the ex-vessel value of all Alaskan fisheries in 2004. After processing, the catch was valued at $1.7 billion. Pacific cod made up 12.5% of the catch, second only to pollock which accounted for 71% of the catch (Hiatt *et al.*, 2005). Kodiak area state waters (within 3 nm of shore) accounted for about 4.5 thousand metric tons of the Pacific cod catch valued at just over $2.5 million (Mattes and Spalinger, 2006).

The relative abundance of Pacific cod and other gadid species in the Gulf of Alaska has fluctuated with interdecadal shifts in sea surface temperatures (Peterson and Schwing, 2003). Because cod are bottom-dwelling and usually found from 50 – 300 m (Allen and Smith, 1988; Love 1991) it seems unlikely that sea-surface temperatures would have a direct effect on their distribution and abundance. Larval cod, however, are pelagic and can be transported via currents into nursery habitats (Garrison and Miller, 1982) and, therefore, are directly influenced by surface temperatures. Changes in surface temperatures may also indirectly affect demersal fish by affecting the distribution and abundance of their predators or prey. In addition, regime shifts have been attributed to changes in primary productivity and can have bottom-up effects that impact the entire ecosystem (see review by Benson and Trites, 2002).

Pacific cod, like most teleost fishes, are heterothermic ectotherms. They exhibit no physiological control of body temperature; therefore, body temperature conforms to and fluctuates with ambient water temperature. This has physiological repercussions for an animal that inhabits an environment with varying ambient water temperature. It is well established that body temperature affects membrane fluidity (Hazel, 1993) and rates of enzyme activity (Guderley and Gawlicka, 1992). Therefore, it is not surprising that in teleost fishes, temperature has been shown for numerous species to impact metabolic rate (*Oncorhynchus nerka*, Brett, 1971; *Alosa sapidissima*, Leonard *et al.*, 1999; *Gadus morhua*, Claireaux *et al.*, 2000), performance during swimming trials (*Oncorhynchus*

nerka, Brett, 1964; *Pleuronectes americanus*, Joaquim *et al.*, 2004; *Oncorhynchus clarki clarki*, MacNutt *et al.*, 2004) and timing of recovery from exhaustive exercise (*Oncorhynchus mykiss*, Kieffer *et al.*, 1994; *Salmo salar*, Galloway and Kieffer, 2003).

Many studies have focused on the effects of water temperature on metabolism of fish (Brett, 1971; Leonard *et al.*, 1999; Claireaux *et al.*, 2000; Gillooly *et al.*, 2001). The metabolic rate of Atlantic cod (*Gadus morhua*) acclimated to 2°C was half of that for fish acclimated to 5°C when the fish were forced to swim to exhaustion (Claireaux *et al.*, 2000). U_{crit} for cutthroat trout acclimated to 7°C was 60% of that for fish acclimated to 14°C (MacNutt *et al.*, 2004). Additionally, Galloway and Kieffer (2003) suggest that recovery of juvenile Atlantic salmon following exhaustive exercise is significantly impeded at 6°C while recovery is quicker at higher temperature (18°C). In addition, temperature effects have been found on anaerobic capacity and post-exercise recovery in the blood of rainbow trout yet no effects were found on recovery in white muscle (Kieffer *et al.*, 1994). The relationship between temperature, swimming performance and recovery is not well defined; clearly more work is needed in this area.

Fish exposed to fluctuating temperatures can either adapt physiologically to the new temperatures or migrate to avoid them. Using trawl surveys over a three year period, Perry *et al.* (1994) illustrate that Pacific cod tend to remain at a relatively fixed temperature between years by vertical migration. In the lab, Claireaux *et al.* (1995) determined that Atlantic cod will avoid changing thermal conditions when possible. In

their study, Atlantic cod were acclimated to 5°C and exposed to a thermally stratified water column ranging from 4-7°C. Cod behaviorally avoided changes in ambient temperature by moving to a preferred temperature zone. Pacific cod may prefer to migrate in order to maintain a constant temperature but migration may not be feasible if the entire habitat is affected by long term or large scale temperature changes.

When a fish is not able to avoid temperature change it may acclimate through changes in its physiology. Changes may include alteration of mitochondrial density (Campbell and Davies, 1978), metabolic rate (Brett, 1964), swimming performance (Taylor *et al.*, 1997), muscle fiber recruitment (Rome *et al.*, 1984) and enzyme activity (Hazel and Prosser, 1974). These changes may take up to several weeks to occur and act to maintain the homeostasis of the organism (see review by Johnston and Dunn, 1987). To maintain organismal homeostasis, changes in metabolic rate must be coupled with commensurate changes in fuel consumption. This has important implications on an organism's ability to survive in a changing environment.

To study the physiological and behavioral effects of temperature on the individual, many studies challenge organisms with exhaustive exercise (Brett, 1964; Kieffer *et al.*, 1994; Taylor *et al.*, 1997; Wilkie *et al.*, 1997). The physiological effects of this protocol are likened to what a fish may experience during predator avoidance, foraging, migration, etc. and, therefore, have relevance to fish behavior in situ. A common method for exhausting a fish is forced exercise in a swim tunnel. This procedure

allows for simultaneous recordings of swimming speed and oxygen consumption during exercise. Forcing fish to swim to exhaustion at different temperatures allows comparisons among swimming performance, metabolic rate, recovery time and various indices of condition. Comparing these factors between fish and at various temperatures allows for a comprehensive description of temperature effects on an organism and gives insight as to how natural temperature fluctuations may affect these organisms in the wild.

Exhaustive exercise is stressful to fish and elicits a myriad of physiological responses. A stressor is any perturbation that disrupts organismal homeostasis. For marine fishes, a stressor can be, but is not limited to, environmental changes, swimming trials and general handling of fish. The initial response to a stressor in fish is the release of catecholamines from the chromaffin cells located in the head kidney (Reid *et al.*, 1996). This response is rapid and catecholamine levels increase immediately after a stressor is applied (Randall and Perry, 1992; Reid *et al.*, 1996). Catecholamines play an important role in exercise physiology because they aid in oxygen delivery to tissues by acting on cardiovascular and respiratory systems (Reid *et al.*, 1998) and serve to mobilize energy stores (Kieffer, 2000). Additionally, catecholamines increase the permeability of cells to Na^+ and K^+ (Horwitz, 1979) and aid in pH regulation by driving Na^+ and Cl^- into red blood cells (Ferguson and Boutilier, 1989; Nikinmaa *et al.*, 1990), thus leading to changes in ion concentrations in the plasma. These plasma ion changes are often measured to determine levels of stress in an organism. Because catecholamines are

released immediately after a stressor, it is difficult to obtain resting levels in the blood without cannulation of the animal.

Cortisol is synthesized in the head-kidney in response to increased stress (Wedemeyer *et al.*, 1990; Barton and Iwama, 1991; Gamperl *et al.*, 1994; Wendelaar Bonga, 1997). Unlike catecholamines, the secretion and therefore presence in the bloodstream is delayed (see review by Wendelaar Bonga, 1997) making it a measurable index of stress response without a need for cannulation of the animal. The cortisol response begins in the hypothalamus with the release of corticotropin-releasing factor, which in turn causes the release of adrenocorticotropic hormone (ACTH) by stimulating the anterior pituitary (Barton, 2002). ACTH acts on the interrenal cells found along on posterior cardinal vein in the head-kidney to synthesize and release cortisol into circulation. Cortisol uptake by cells is thought to occur via a carrier protein. Once in the cell the hormone is either bound to a receptor and activated or is metabolized and, thus, deactivated (see reviews by Wendelaar Bonga, 1997 and Mommsen *et al.*, 1999). Similar to catecholamines, cortisol aids in the mobilization of energy stores and has been shown to stimulate release and synthesis of glucose thus resulting in increased concentrations of plasma glucose (Gamperl *et al.*, 1994; Mommsen *et al.*, 1999). Cortisol levels may remain elevated for several hours (Milligan, 1996) or days to weeks (Haukenes and Buck, 2006) following exposure to a stressor. In fact, cortisol levels in fish do not typically reach their highest concentrations until 1-2 hours after exhaustion (Gamperl *et al.*, 1994).

Cortisol also influences lactate metabolism and glycogen resynthesis subsequently slowing recovery from a stressor (Milligan, 1996). When cortisol synthesis is blocked, rates of lactate clearance and glycogen resynthesis are increased (Pagnotta *et al.*, 1994). It seems counterintuitive that an organism would slow its own recovery; however, it may be that slowing the rate of the recovery process ultimately conserves energy. Lactate clearance by way of glycogen resynthesis requires the expenditure of energy. Arguably, there is no reason to store energy in the form of glycogen at this point. It is energetically more efficient to convert lactate to pyruvate and shunt it into the tricaboxylic acid cycle by way of acetyl CoA and subsequently generate ATP. This avoids the additional energy that would be expended to clear the lactate pool through anabolic processes rather than simply allowing it to remain for use in aerobic metabolism. In contrast to other studies of recovery of fish from exhaustive exercise, Milligan *et al.* (2000) found that cortisol does not increase in rainbow trout (*Oncorhynchus mykiss*) that are allowed to swim at low velocity after exhaustive exercise but does increase if the fish are placed into still water after exhaustion. It may be that, even though fish must take in more oxygen to swim, oxygen is more readily available to a fish in moving rather than static water. This suggests that the post-exercise increase in cortisol may *not* be related to the exercise at all but rather a response to environmental and/or experimental conditions.

Hematocrit, packed red blood cell volume, is known to affect oxygen carrying capacity in fish (Virtanen and Oikari, 1984) and has been shown to impact swimming performance in rainbow trout (Pearson and Stevens, 1991). Changes in hematocrit may

result from splenic release of erythrocytes, which has been shown to occur following exhaustive exercise in rainbow trout (Wells and Weber, 1990). In addition hematocrit is often used as an indicator of osmotic stress in fish (Handeland *et al.*, 1996) as water movement may cause erythrocyte swelling (Nikinmaa, 1983; Wells and Weber, 1990) and hemoconcentration or an increase in concentrations of cells due to water loss (Wood *et al.*, 1983). In exhaustive exercise trials, rainbow trout demonstrate and almost 50% increase in hematocrit which does not return to resting levels even after 4 hours post-exercise (Wang *et al.*, 1994).

Stress can also affect ionic and osmotic balance in fish by driving ions and water in and out of cells as well as in and out of the fish itself. In marine fish, electrolytes are gained and water is lost across the gills even after exhaustion (Wood, 1991). In freshwater rainbow trout, fluid moves into cells due to the increased levels of intracellular lactate (Milligan and Wood, 1986). In addition, hemoconcentration occurs and plasma electrolyte concentration increases due to the influx of water into cells (Wood, 1988). Water movement can lead to changes in hematocrit as well as ion (Redding and Schreck, 1983) and total protein concentrations in the blood (Wang *et al.*, 1994). Because teleosts occur in either a hypotonic (freshwater) or hypertonic (marine) environment, stress affects ionic and osmotic balance differently in marine fish than freshwater fish. After exhaustive exercise, recovery in marine acclimated trout takes approximately half the time as freshwater acclimated trout and is most likely due to the fact that marine trout can excrete H^+ at five times the rate to the environmental water than their freshwater

counterparts (Tang *et al.*, 1989). This increased ability to excrete H^+ may be due to physiological differences associated with marine acclimation or the higher concentrations of various ions in seawater. In addition, the branchial epithelium of marine teleosts has a higher permeability to ions than that of freshwater teleosts (Evans, 1979), thus allowing for faster recovery. Efflux of Na^+ in marine acclimated rainbow trout is ten fold that of freshwater rainbow trout (Greenwald *et al.*, 1974). Acidosis is prevented by the movement of strong ions which in turn causes H^+ to accumulate either inside or outside of the cell. Early in recovery, Na^+ influx is stimulated while Cl^- influx is inhibited causing a net movement of H^+ out of the cell, later in recovery this is reversed thus causing a net movement of H^+ into the cell (Milligan *et al.*, 1991).

In two experiments, the hypothesis that acclimation temperature affects physiological parameters in Pacific cod, specifically metabolism and swimming performance was tested. To do this, critical swimming speed, rates of oxygen consumption while swimming and rates of recovery after exhaustive exercise of cod held at different temperatures were determined. In addition, a variety of blood parameters were measured to illustrate the effect of swim trials on the condition of fish. It was predicted that fish held and tested at the different temperatures would differ in their swimming performance, metabolic rate and recovery rate after exhaustion. Specifically, it was predicted that fish acclimated to higher ambient temperatures would attain greater swimming speeds and higher rates of oxygen consumption and faster recovery than fish acclimated to lower ambient temperatures.

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Chapter 1. Temperature effects on swimming performance and metabolic rate of Pacific cod (*Gadus macrocephalus*)[1]

Critical swimming speeds (U_{crit}) and rates of oxygen consumption of Pacific cod (*Gadus macrocephalus*) acclimated to two different temperatures were determined by exhaustive exercise in a Brett-type swim tunnel. Blood samples collected before and after swimming were analyzed for hematocrit and plasma concentrations of cortisol, metabolites and ions to assess the impact of fish condition on swimming performance. Fish acclimated and swum at 4°C did not differ significantly in either U_{crit} (1.07 BL/s) or resting metabolic rate (35.34 mg $O_2/kg^{0.8}$/hr) from those acclimated and swum at 11°C (1.06 BL/s; 49.43 mg $O_2/kg^{0.8}$/hr). Plasma concentrations of cortisol, glucose and lactate increased significantly from pre- to post-swim, yet only cortisol differed significantly between temperature groups. Higher concentrations of cortisol in the lower temperature group suggest a stress associated with this temperature. The few significant differences detected between temperature groups suggests that the temperatures utilized in this study have little impact on swimming performance or metabolic rate in Pacific cod.

Key words: Pacific cod; swimming; metabolic rate; temperature; U_{crit}; exhaustion.

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Introduction

Environmental conditions can exert profound effects on the physiology of an organism and are often manifest as changes in metabolism and ultimate survival. For marine fishes, abiotic parameters that strongly influence physiology include salinity (Claireaux *et al.*, 1995), temperature (Johnston and Dunn, 1987), oxygen tension (Claireaux *et al.*, 1995), toxins (Beyers *et al.*, 1999) and a myriad of other factors. A changing environment may impact the energy requirements for necessary activities such as predator-prey interactions, migration, reproduction, etc. and can affect the fitness and overall survival of an organism.

Because locomotion impacts all aspects of the life history of fishes, swimming performance has been hypothesized to provide a reliable indicator of the effects of both biotic and abiotic factors on the condition of the individual (Dalla via *et al.*, 1989; Kieffer, 2000; Martinez *et al.*, 2003). Although swimming performance can be assessed a number of different ways such as sprint speed, rate of acceleration or endurance swimming (see review by Wood, 1991), critical swimming speed (U_{crit}) is an accepted evaluation of prolonged swimming performance (Beamish, 1978) and has been used widely to determine the effects of environmental changes on the swimming ability of fish (Farrell *et al.*, 1998; Lee *et al.*, 2003; MacNutt *et al.*, 2004). In general, swim trials used to assess U_{crit} force a fish to swim in a flume against an increasing current for pre-set durations until it is exhausted and can no longer maintain its position in the flume. U_{crit} is

calculated as the final speed in which the fish swam the entire duration in addition to the portion of the next speed it swam.

Repeated determination of U_{crit} has been used to assess the general health or condition of a fish (Jain *et al.*, 1998; Farrell *et al.*, 1998). In repeat swim trials, fish are forced to swim to exhaustion, allowed to rest for a specified period of time and then forced to swim to exhaustion again. The first U_{crit} (U_{crit1}) is compared to the second U_{crit} (U_{crit2}) calculate a recovery ratio, U_{crit2}/ U_{crit1} (Jain *et al.*, 1998). A recovery ratio of ≥ 1 indicates that the fish had sufficient time to recover from the first swim trial while a recovery ratio <1 suggests that the fish did not have sufficient time to recover.

The aerobic and anaerobic capacity of a fish determines the point at which a fish fails during exercise due to insufficient ATP production, depletion of glycogen reserves and ionic disturbances (Wood, 1991). At this point, metabolic wastes are accumulated more rapidly than they can be cleared resulting in a disruption of organismal homeostasis and the fish succumbs to exhaustion. A fish is considered exhausted when it can no longer perform burst or sprint activity although it may be able to swim at slow speeds (Milligan, 1996).

Swimming performance, or U_{crit}, is dependent upon the condition of the animal and the environmental conditions in which the exercise is conducted. For example, fish are known to exhaust more rapidly during times of fasting (Martinez *et al.*, 2003) or

when oxygen concentrations (Davis *et al.*, 1963) or temperature (MacNutt *et al.*, 2004) are outside of the fish's optimal range. Atlantic cod (*Gadus morhua*) demonstrate a 30% decrease in swimming ability when starved for 16 weeks prior to swim trials (Martinez *et al.*, 2003). Chinook salmon (*Oncorhynchus tshawytscha*) showed decreases in swimming performance of up to 30% when faced with low oxygen concentrations (Davis *et al.*, 1963). Smallmouth bass (*Micropterus dolomieu*) are able to increase maximum sustained swimming speed 4.5 fold with an increase in temperature from 5 to 30°C (Larimore and Duever, 1968).

Most fish are heterothermic ectotherms and thus their body temperature fluctuates with ambient water temperature. Temperature impacts the rate of biochemical reactions in a Q_{10} dependent fashion and exerts influence systemically leading to changes in whole body processes such as metabolic rate and swimming performance. However, temperature effects on these parameters are not consistent across species or studies. For example, Claireaux *et al.* (2000) detail a 2.7 fold increase in both standard metabolic rate (SMR) as well as active metabolic rate (AMR) when Atlantic cod are exposed to an 8°C increase in temperature (from 2 to 10°C). Schurmann and Steffensen (1997) detail a 2.2 fold increase in SMR but only a 1.4 fold increase in AMR when Atlantic cod are exposed to a 10°C increase in temperature (from 5 to 15°C). Additionally, He (1991) found that the U_{crit} of Atlantic cod at 0°C are about half that of those at 5°C, whereas Schurmann and Steffensen (1997) indicate only a 1.2 fold increase in U_{crit} (speeds reaching just below 2 BL/s) with a 10°C increase in temperature. Increasing temperature from 5 to

15°C increases SMR in young sockeye salmon (*Oncorhynchus nerka*) four fold and also doubles AMR when the fish were forced to swim to exhaustion (Brett, 1964). Sockeye show a 1.3 fold increase in U_{crit} when temperatures are increased from 5 to 15°C, yet swimming speeds reach up to 4.1 BL/s (Brett, 1964), much higher than that reported for Atlantic cod. Although species differences exist with respect to temperature and metabolic performance, generally metabolism and swimming speed increase with increasing temperature in teleosts.

Pacific cod (*Gadus macrocephalus*) are distributed from the Yellow Sea to the Bering Strait and are found as far south as Los Angeles, CA. They are usually found on the continental shelf and upper slope and mainly live between 100-400 m depth (Cohen *et al.*, 1990). In the Gulf of Alaska, Pacific cod inhabit a region that is exposed to intra- and interannual variability in sea surface temperature as well as interdecadal variation in climate defined by the Pacific Decadal Oscillation (see review by Mueter, 2004). Interdecadal climate shifts have been associated with fluctuations in Pacific cod distribution and abundance as well as that of other groundfish (Hare and Mantua, 2000). It is not known how climate variability affects Pacific cod distribution and abundance or how temperature changes during this climate change affect Pacific cod physiology.

To determine the effect of temperature on swimming performance and metabolic rate, wild-caught adult Pacific cod were acclimated to either 4 or 11°C. These temperatures were chosen as they were slightly above and below capture temperature (7-

9°C). Following acclimation, fish were forced to swim to exhaustion. During the swim trials, individual rates of metabolism, U_{crit} and U_{crit} ratio were determined. Prior to and immediately following the swim trials, fish were sampled for blood that was later assayed for hematocrit and concentrations of cortisol, lactate, glucose, total protein and specific ions as indicators of condition. These parameters were compared between temperature treatments. It is believed that acclimation to the different temperature treatments will result in significant differences in all parameters measured.

Materials and Methods

Adult Pacific cod were caught in June 2005 in waters surrounding Kodiak Island (57°45' N 152°15' W) with cod pots (145 x 130 x 70 cm). Pots were baited and set at approximately 20 m depth and allowed to soak for 2-3 days prior to collection. Fish were transported back to the Kodiak Fisheries Research Center on Kodiak Island. Upon arrival in the wet lab, they were measured for total length on a measuring board (± 1.0 cm) and weighed (± 0.01 kg). Maximum width and height were measured using a caliper just posterior to the pectoral fins (±0.1 mm). Each fish was marked using a unique color and number coded tag (T-bar tag FD-68B, Floy Tag®, Seattle, Washington) prior to being placed into four 22 m^3 tanks supplied with sand filtered seawater drawn from Trident Basin at a depth of 25 m and at an average temperature of 11.8 ± 0.6°C between the months of July and September of 2005. Two of the tanks were chilled to a low temperature (3-5°C) and two other were slightly chilled to a high temperature (10-12°C).

Fish were allowed to acclimate for at least two-weeks in the laboratory prior to any further handling or testing. During this time fish were fed Pacific herring (*Clupea pallasii*) to satiation every three days.

Sixteen fish in two temperature treatments (8/group) were used to evaluate the general metabolic response of Pacific cod to different temperatures. The low temperature group was acclimated to 3-5°C and the high temperature group to 10-12°C. The low temperature group consisted of four males and four females with a mean mass of 1.43 ± 0.18 kg (range 0.76-2.16 kg) and total length of 51.4 ± 2.2 cm (range 43-59 cm). The high temperature group consisted of six males and two females with a mean mass of 1.57 ± 0.22 kg (range 0.88-2.42 kg) and total length of 51.1 ± 2.3 cm (range 44-60 cm). Neither mass nor total length was significantly different between groups (t=-0.504, df=14, P=0.622 and t=0.0775, df=14, P=0.939, respectively).

An initial blood sample was collected from each fish after the initial two-week acclimation period. Each fish was captured from the tanks using a low abrasion dipnet and immersed in a 30 L solution of anesthetic (tricaine methansulfonate; 100 ppm) for up to two minutes. Once anesthetized, each fish was placed on a V-board and 0.5 ml of blood was collected from the caudal vasculature using a 21 gauge needle and 3 ml syringe. Individual blood samples were placed into labeled vials coated with and anticoagulant, ammonium heparin, for the preparation of plasma samples. Hematocrit of each sample was determined in duplicate by drawing blood into micro-hematocrit

capillary tubes (Fisherbrand®) following centrifugation at 12,000 RPM for 10 minutes (Unico® Micro-hematocrit). The remainder of the blood sample was refrigerated immediately (0-1°C) and was separated via centrifugation (2500 rpm for 15 minutes, Fisher Scientific accuSpin™ Micro R) ≤30 min of collection. Plasma was drawn off using a long-stemmed Pasteur pipette and placed into a labeled vial and stored at -50°C for later analysis. Fish were again measured for total length, standard length, width, height and weight as described above and returned to their respective tanks for an average of 48 days prior to swim trials (time ranged from 30-75 days).

Immediately preceding the swim trials, individual fish were fasted for either four days (high temperature group) or five days (low temperature group) to minimize the effect of digestion on metabolism. Fish were captured from tanks using a low abrasion dipnet and placed into a 370 L Brett-type swim flume maintained at the acclimation temperature of the fish. The swimming section of the flume was constructed of Lexan® and had a length of 1.87 m and a diameter of 20 cm. Width, height and total length measures were used to correct water velocities for solid blocking effects (Bell and Terhune 1970) during the swim trials. Prior to initiating the swim trial, fish were trained and acclimated to the swim flume to familiarize them with the swim protocol and to teach them to maintain position in the flume. Implementation of this procedure tended to discourage the fish from resting at the back of the tunnel. Training involved exposing the fish to minor increases and decreases in current velocity for 5-10 minutes. Water velocity was then systematically increased in a stepwise fashion at 0.2 body lengths per second

(BL s^{-1}) each five minutes until the fish was unable to swim away from the rear of the swim tunnel. Water velocity was then decreased to 0.5 BL s^{-1} and the fish was allowed to rest for five minutes. This process was repeated once more and then the fish was allowed to acclimate to the flume at a water velocity of 0.5 BL s^{-1} for 36 h. During acclimation, water temperature (acclimation temperature ± 1.0°C) and percentage dissolved oxygen (>85%) were monitored using a probe (YSI 600R multiparameter water quality sonde) and maintained by periodically adding new seawater as needed.

During swim trials, water velocity was systematically increased from rest (0.5 BL s^{-1}) to exhaustion and the rate of oxygen consumption was simultaneously measured. U_{crit1} was determined by increasing water velocity by 0.1 BL s^{-1} every 0.5 h until the fish was exhausted. The fish was considered exhausted when it rested against the grate at the back of the swim tunnel for ≥10 seconds. Once exhausted, water velocity was decreased and maintained at 0.5 BL s^{-1} for one hour. A second critical swimming speed (U_{crit2}) was then determined in the same manner as for U_{crit1} and an index of recovery was calculated from U_{crit2}/ U_{crit1} (Jain *et al.*, 1998). Immediately after determination of U_{crit2}, fish were removed from the swim flume, anaesthetized, sampled for 1 ml of blood, weighed and measured (as above). Following each trial, the oxygen concentration in the swim flume in the absence of a fish was measured for one hour to determine the biological activity of the water to correct rates of oxygen consumption obtained during a swim trial.

Rate of oxygen consumption, corrected for fish weight ($VO_{2\ Ws}$; mg $O_2/kg^{0.8}/hr$) was calculated from oxygen depletion in the swim tunnel at each velocity of the swim trial according to the following equation adapted from Newell *et al.* (1977):

$$VO_{2\ Ws} = (1/W)^{0.8} * VO_2\ (exp) - B$$

where W is the fish mass (kg), VO_2 (exp) is the average oxygen depletion (mg O_2) per minute in the swim tunnel during a specific swimming speed, and B is the oxygen depletion in the swim tunnel per minute without the experimental animal present. Oxygen depletion was determined from the slope of the oxygen concentration in the swim tunnel regressed as a function of time (minutes) for each time step. Masses were raised to the 0.8 power to address the allometric relationship between mass and metabolic rate (Reidy *et al.*, 1995). Resting metabolic rate (RMR) was determined by extrapolating oxygen consumption data to 0 BL/s using an exponential curve (Figure 1.1; Brett, 1964; Reidy *et al.*, 2000). SigmaPlot 2004 version 9.0 (Systat Software, Inc.) was used to fit a two parameter exponential curve to the oxygen consumption data. Q_{10} was calculated based on the extrapolated RMR.

Plasma was assayed for concentrations of glucose, lactate and protein using a SPECTRAmax® 340pc384 microplate spectrophotometer (Molecular Devices, Sunnyvale, CA), SOFTmax® PRO software (Molecular Devices) and enzymatic assay kits (Glucose: Wako Pure Chemical Industries, Richmond, VA; Lactate: Trinity Biotech,

St. Louis, MO; Protein: Pierce, Rockford, IL) according to manufacturers protocols adapted for use in 96-well microplates. Samples were assayed in triplicate to determine a mean optical density. Solutions of known concentrations of each constituent were also assayed on every plate to determine the linear relationship between optical density and concentration of the specific constituent. The mean intra-assay variation for the glucose, lactate and protein assays were 5.5%, 4.6% and 4.7%, respectively and the mean inter-assay variations were 2.3%, 2.5% and 3.6%, respectively.

Plasma sodium, chloride and potassium concentrations were determined using a Medica EasyElectrolytes ion meter (Medica Corporation, Bedford, MA). Samples were diluted with deionized water (11:5) to fit plasma concentrations into the working range of the instrument. All samples were assayed in duplicate. The mean intra assay variation was 0.2%.

Concentrations of cortisol in plasma were determined using standard radioimmunoassay techniques originally described by Foster and Dunn (1974) and further modified by Redding *et al.* (1984). Samples were assayed in duplicate and the intra assay variation was <5%, inter assay variation was <10%. Briefly, 10 µl of sample was diluted with 200 µl glutamate buffer and heated in a water bath at 90-100°C for 15 minutes. Once cooled to room temperature, 0.04 µCi d ^{3}H cortisol in 500 µl phosphate buffer (pH 7.6) was added and whirl-mixed. A cortisol-specific antiserum diluted to bind 50% of the radio-labeled cortisol was then added and whirl-mixed. Samples were placed

in an ice bath for five-minutes at which point ice-cold 2.5% dextran-coated charcoal was added and the mixture was whirl-mixed. Samples were left in the ice bath for another five minutes before centrifugation. The supernatant was then decanted into individual vials containing scintillation cocktail. A standard curve was generated and used to calculate cortisol concentrations. Known concentrations of cortisol added to dextran-charcoal stripped Pacific cod plasma ran parallel with the standard curve.

Oxygen consumption data were analyzed using two-way repeated measures analysis of variance with temperature and swim speed as factors. Blood parameters were analyzed using two-way repeated measures analysis of variance with temperature and pre- or post- swim (time) as factors. Specific differences were determined using the Holm-Sidak method. A *t*-test was used to test for differences in U_{crit} between temperature treatments. SigmaStat 3.0 statistical software (Systat Software, Inc.) was used for all statistical analyses. All tests were performed at $\alpha=0.05$. Data are presented as mean ± standard error of measure.

Results

The results of swim trials performed on eight low temperature and eight high temperature acclimated fish are summarized in Figure 1.1 and Figure 1.2. Mean rate of oxygen consumption increased exponentially with swimming speed in both the low ($y=29.01e^{0.18x}$, $R^2=0.92$, $P<0.001$) and high ($y=49.23e^{0.13x}$, $R^2=0.97$, $P=0.004$)

temperature groups during the first swim (Figure 1.1). U_{crit1} averaged 1.07 ± 0.03 BL s^{-1} in the low temperature group and 1.07 ± 0.05 BL s^{-1} in the high temperature group and did not differ significantly either between temperature treatments (t=0.007, df=14, P=0.994) or sex of the fish (F=0.746, df=1, P=0.405). U_{crit2} averaged 1.06 ± 0.03 BL s^{-1} in the low temperature group and 1.07 ± 0.05 BL s^{-1} in the high temperature group and did not differ significantly either between temperature treatments (t=-0.0882, df=14, P=0.931) or from U_{crit1} in the low temperature (t=0.292, df=14, P=0.774) or high temperature group (t=0.0994, df=14, P=0.992).

The rate of oxygen consumption for Pacific cod increased significantly with increased swim speed during the first swim (F=60.160, df=5, P<0.001) but did not significantly differ between temperature groups (F=1.280, df=1, P=0.277). During the second swim the rate of oxygen consumption did not significantly differ with swim speed (F=1.685, df=5, P=0.151) or between temperature groups (F=0.297, df=1, P=0.595). Fish acclimated to high temperature tended to have higher rates of oxygen consumption at each step during the first swim than low temperature fish and were about 14% higher, overall. Fish in the high temperature group also tended to have a higher RMR than fish in the low temperature group in the first swim (49.43 ± 6.68 mg O_2 $kg^{-0.8}$ hr^{-1} vs 35.34 ± 8.18 mg O_2 $kg^{-0.8}$ hr^{-1}, respectively). The Q_{10} for these fish was calculated to be 1.8 based on extrapolated RMR from the first swim.

The results of blood data (hematocrit readings and cortisol, glucose, lactate, total protein and ion assays) are summarized in Figure 1.3. Analysis of hematocrit data revealed a significant interaction between temperature and time (F=21.117, df=1, P<0.001). Hematocrits increased from pre-swim (23.58 ± 0.91 %) to post-swim (31.07 ± 1.16 %) in the high temperature group but showed a slight decrease from pre- (29.00 ± 0.59 %) to post- (27.75 ± 1.26 %) swim in the low temperature group. In addition, fish in the low temperature group had significantly higher hematocrits pre-swim than those in the high temperature group. This relationship was reversed post swim.

Concentrations of plasma cortisol were significantly higher in the low temperature group (pre-swim: 75.06 ± 11.85 ng/ml; post-swim: 181.85 ± 14.88 ng/ml) than in the high temperature group (pre-swim: 20.96 ± 6.42 ng/ml; post-swim: 158.26 ± 16.66 ng/ml) throughout the experiment (F=5.154, df=1, P=0.038) and increased significantly in both groups from pre-swim to post-swim (F=94.216, df=1, P<0.001). Acclimation temperature had no significant affect on concentrations of plasma glucose (F=5.154, df=1, P=0.706), lactate (F=1.592, df=1, P=0.224) or total protein (F=0.004, df=1, P=0.953). Concentrations of plasma glucose increased significantly from pre- (low temperature: 3.26 ± 0.22 mmol/L; high temperature: 2.85 ± 0.17 mmol/L) to post-swim (low temperature: 5.08 ± 0.45 mmol/L; high temperature: 5.21 ± 0.71 mmol/L) in both temperature groups (F=13.932, df=1, P=0.003). Concentrations of plasma lactate also increased significantly from pre- (low temperature: 0.61 ± 0.09 mmol/L; high temperature: 0.76 ± 0.16 mmol/L) to post-swim (low temperature: 4.79 ± 0.46 mmol/L;

high temperature: 3.78 ± 0.64 mmol/L) in both temperature groups (F=25.077, df=1, P<0.001). Similarly, total plasma protein increased significantly from pre-swim (low temperature: 15334.28 ± 808.66 μg/ml; high temperature: 15302.77 ± 992.79 μg/ml) to post-swim (low temperature: 19966.55 ± 586.04 μg/ml; high temperature: 20104.10 ± 1340.97 μg/ml) in both groups (F=19.423, df=1, P=0.001).

Analysis of plasma Na^+ concentrations revealed a significant interaction between temperature and time (F=13.236, df=1, P=0.004). Concentration of Na^+ in the plasma increased from pre- (169.39 ± 1.73 mmol/L) to post-swim (184.08 ± 3.38 mmol/L) in low temperature acclimated fish; however, no change was observed between pre- (168.41 ± 1.56 mmol/L) and post- (166.79 ± 1.34 mmol/L) swim samples in the high temperature acclimated fish. Plasma K^+ concentrations did not significantly differ between temperature groups (F=2.278, df=1, P=0.148) and did not significantly change from pre to post swim (F=0.554, df=1, P=0.472). Plasma Cl^- concentrations were significantly greater in low temperature (pre-swim: 158.15 ± 1.72 mmol/L; post-swim: 163.71 ± 3.70 mmol/L) than high temperature (pre-swim: 154.29 ± 1.83 mmol/L; post-swim: 147.64 ± 1.28 mmol/L) fish (F=17.281, df=1, P<0.001) and did not significantly change from pre- to post-swim (F=0.045, df=1, P=0.835).

Discussion

Measurements of oxygen consumption during exhaustive swimming trials of Pacific cod indicate that fish acclimated and exercised at 4°C consume similar amounts of oxygen and perform just as well as Pacific cod acclimated and exercised at 11°C. Blood samples taken before and after swims suggest slight osmotic differences between temperature groups but few differences in metabolites. These results suggest that the acclimation temperatures chosen have little effect on metabolic rate and swimming performance.

It is generally accepted that metabolic rate and swimming performance are dependent upon temperature and increase with increasing temperature up to a point, after which metabolic rate and performance decrease (Brett, 1971; Beamish, 1978). The point of optimum performance seems to be related to an optimum temperature (Brett, 1971). Although the optimum temperature for Pacific cod egg survival has been found to be 3-5°C (Alderdice and Forrester, 1971), optimum temperature for adults is loosely described as being <10°C (Love, 1996). Catch studies indicate that Pacific cod preferentially inhabit waters slightly above 7°C (Perry *et al.*, 1994). Temperature recorders placed in pots during the collection of fish used in this study indicate that these fish were in water of approximately 7.5-9°C. If their optimum range is between 7-9°C then the temperatures chosen in this experiment were on either side of that optimum (Figure 1.4). This may

help to explain the lack of significant differences seen in metabolic rate and swimming performance between temperature groups, which may peak between 7-9°C.

Higher concentrations of cortisol found in the low temperature fish may have increased the metabolic rates of these fish to levels not significantly different from those of warm fish. The effects of cortisol as a glucocorticoid as well as a mineralocorticoid are well documented as well as the hormone's effects on carbohydrate, lipid and protein metabolism (see review by Mommsen *et al.*, 1999). Cortisol acts to mobilize fuel stores to maintain homeostasis and therefore has direct effects on metabolism (van der Boon *et al.*, 1991). Therefore, increases in cortisol would have effects on metabolism and could increase rates of oxygen consumption (Mommsen *et al.*, 1999).

Changes in Pacific cod abundance concomitant with interdecadal climate shifts in the Gulf of Alaska (Hare and Mantua, 2000) may be due to the effects of thermal stress as indicated by higher circulating levels of cortisol at low temperatures. Although low temperatures may not be the primary stressor that elicits the decline in Pacific cod, low temperatures may reduce the rate at which fish can recover from a stressor and this may be mediated by cortisol (Milligan, 1996). It is interesting to note, however, that even though the low temperature acclimated fish had higher concentrations of plasma cortisol and were likely more stressed, their swimming performance was not significantly different from the warm acclimated fish. It may be that this level or type of stressor does not hinder swimming performance. Similar results have been found with salmonids in

which a three-fold elevation in cortisol levels did not affect swimming performance (Gregory and Wood, 1999).

Previous studies have examined the effects of chronically elevated cortisol in rainbow trout and found that cortisol levels of 100-200 ng/ml over a 10 day period cause a decrease of liver glycogen while plasma lactate, protein and glucose were not significantly different from controls (Andersen *et al.*, 1991). Similarly, plasma lactate, protein and glucose concentrations did not differ between temperature groups in this study, even though low temperature acclimated fish had higher plasma cortisol concentrations. Because glycogen concentrations were not measured in this study, the fate of tissue glycogen in Pacific cod is unknown. In the future, it would be beneficial to measure tissue glycogen concentrations before and after swims by taking muscle samples prior to and immediately after swimming because decreased levels may be a further indicator of stress. Moreover, glycogen reserves can contribute significantly to the swimming performance of a fish (see review by Beamish, 1978).

Although metabolites did not differ between temperature groups, plasma Cl^- concentrations were greater in low temperature fish post-swim than that of high temperature fish. Increased plasma ion concentrations are indicative of an osmotic stress (Redding and Schreck, 1983). Similarly, Na^+ concentrations were slightly higher in low temperature acclimated fish, yet an interaction between temperature and time masked any significant differences. Redding and Schreck (1983) also indicate decreases in hematocrit

associated with stress, yet, once again, an interaction between variables masked any differences in this study. However, hematocrit data in this study suggests that low temperature acclimated fish were stressed prior to swim trials. High temperature acclimated fish show increases in hematocrit from pre- to post-swim samples indicating splenic release of red blood cells (Wells and Weber, 1990) yet no change in hematocrit is observed in the low temperature group from pre- to post-swim samples suggesting that these fish utilized red blood cell reserves prior to swimming. The increases in concentrations of plasma lactate and glucose in association with exercise has been seen in other studies (Milligan and Wood, 1986; Wood *et al.*, 1990), however, the increase in plasma protein concentrations is indicative of fluid moving out of the extracellular fluid into the white muscle (Wang *et al.*, 1994) or water moving out of the fish itself and suggests an osmotic stress in both temperature groups. Further work is needed to determine if low temperature acclimated Pacific cod are in fact osmotically stressed and how this affects them physiologically.

Temperature acts on physiology in a Q_{10} dependent fashion and is evidenced by an increase in biochemical reactions and ultimately in metabolic rate. Although Q_{10} differs among temperatures and species, a Q_{10} between 2 and 3 is expected for fish (Schurmann and Steffensen, 1997; Claireaux *et al.*, 2000). In this study, the impact of acclimation temperature on metabolic rate of Pacific cod was not significant and the Q_{10} was below 2, suggesting that the temperatures chosen in this study were outside of the optimum for these fish (Figure 1.4) or that these fish acclimate to maintain metabolic rate

when exposed to fluctuating temperatures as those seen in interdecadal climate changes. This is important when determining the energy these fish need to survive.

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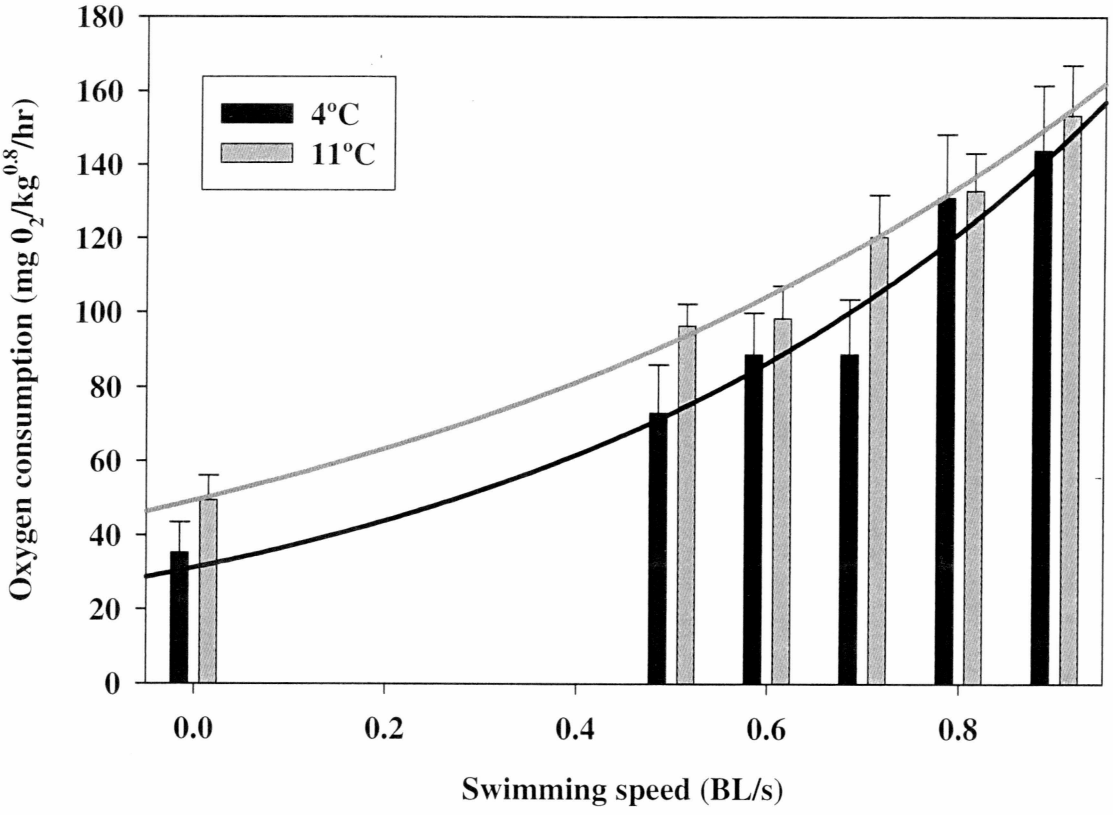


Figure 1.1. Mean oxygen consumption ± *SE* of Pacific cod during the first swim. Fish were acclimated to 4°C (low temp) or 11°C (high temp) and swum at increasing swimming speeds. Note: rates of oxygen consumption at 0 BL s^{-1} are extrapolated values and are indicative of RMR (see methods).

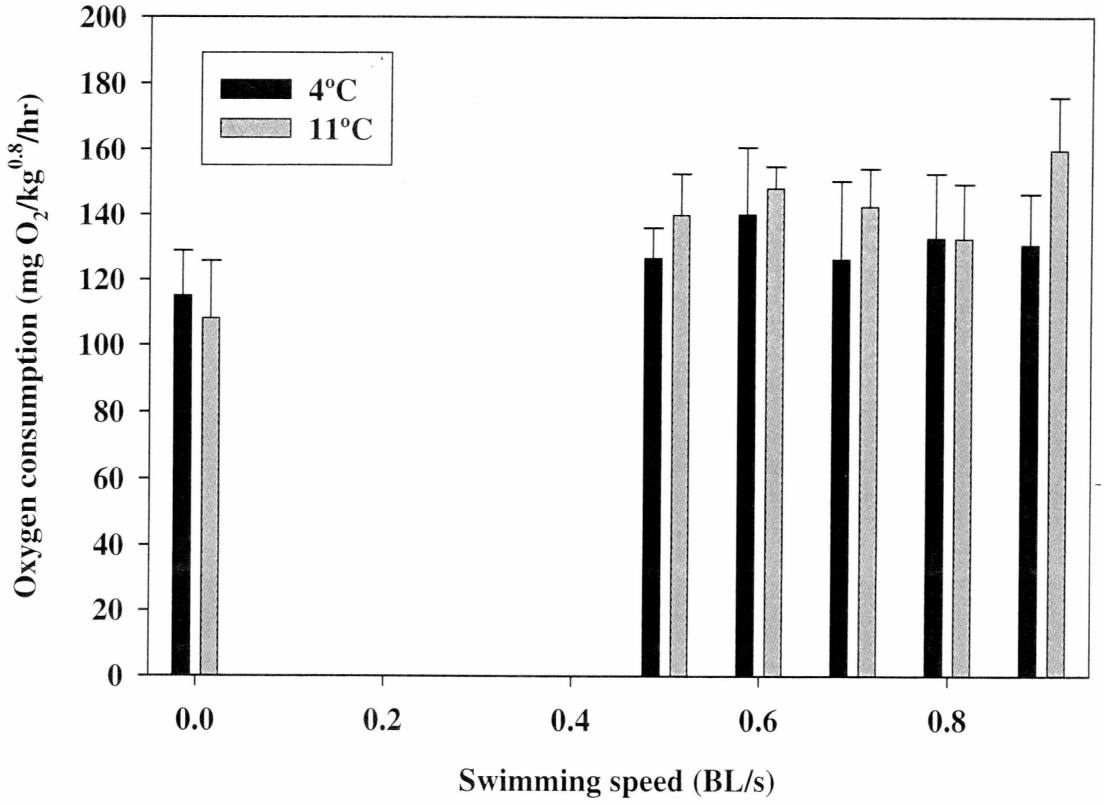
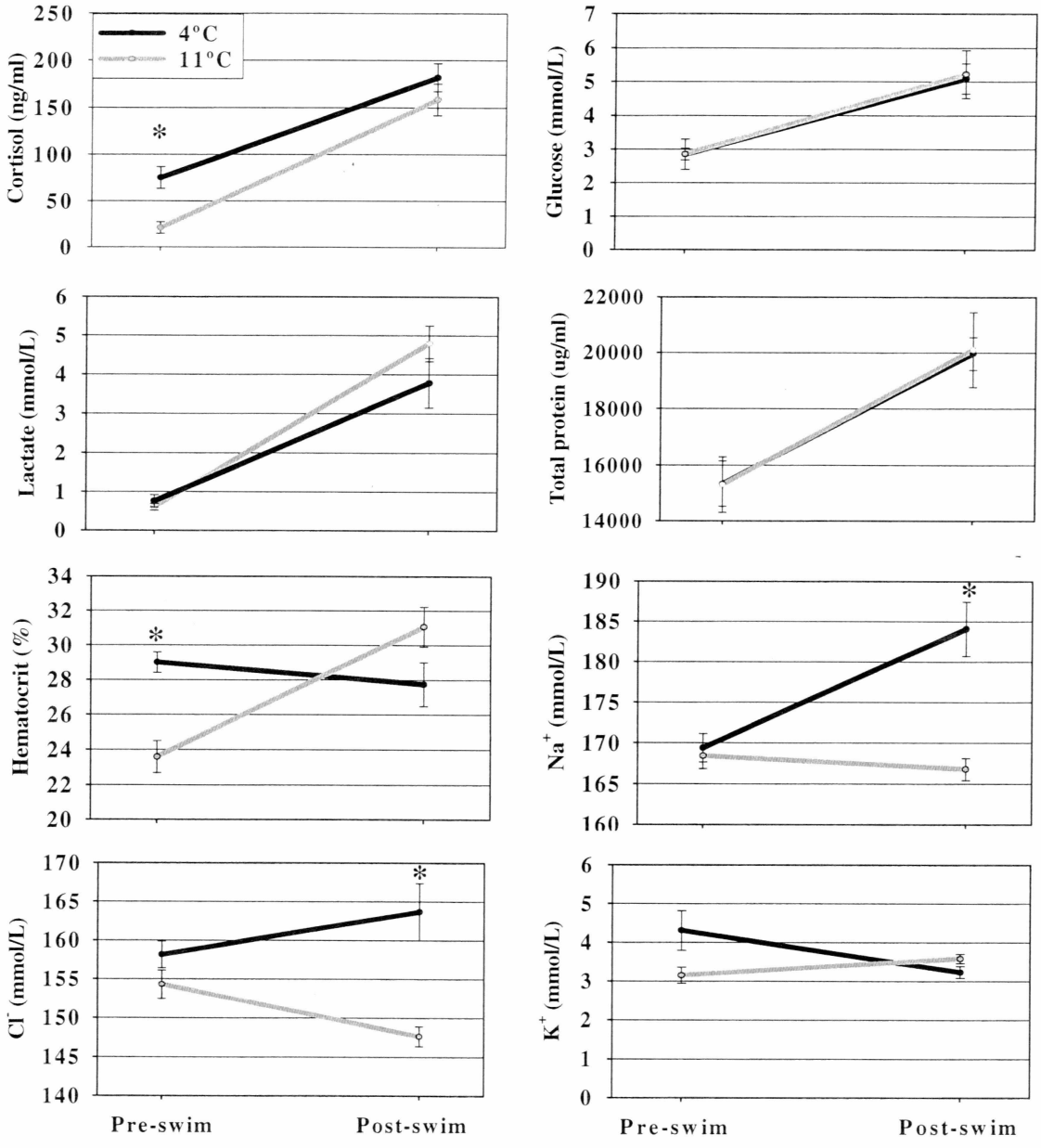


Figure 1.2. Mean oxygen consumption ± *SE* of Pacific cod during the second swim. Fish were acclimated to 4°C (low temp) or 11°C (high temp) and swum at increasing swimming speeds. Note: rates of oxygen consumption at 0 BL s^{-1} are extrapolated values and are indicative of RMR (see methods).

Figure 1.3. Blood constituents ± *SE* for Pacific cod acclimated to 4 and 11°C. Blood samples were taken prior to swimming as well as immediately after exhaustion. A two-way repeated measures ANOVA revealed significant interactions between temperature and swim state for hematocrit (F=21.117, df=1, P<0.001) and Na^+ concentrations (F=13.236, df=1, P<0.004). Significant differences between temperature groups are denoted by "*".


4°C
11°C
Cortisol (ng/ml)
Glucose (mmol/L)
Lactate (mmol/L)
Total protein (ug/ml)
Hematocrit (%)
Na+ (mmol/L)
Cl- (mmol/L)
K+ (mmol/L)
Pre-swim
Post-swim
Pre-swim
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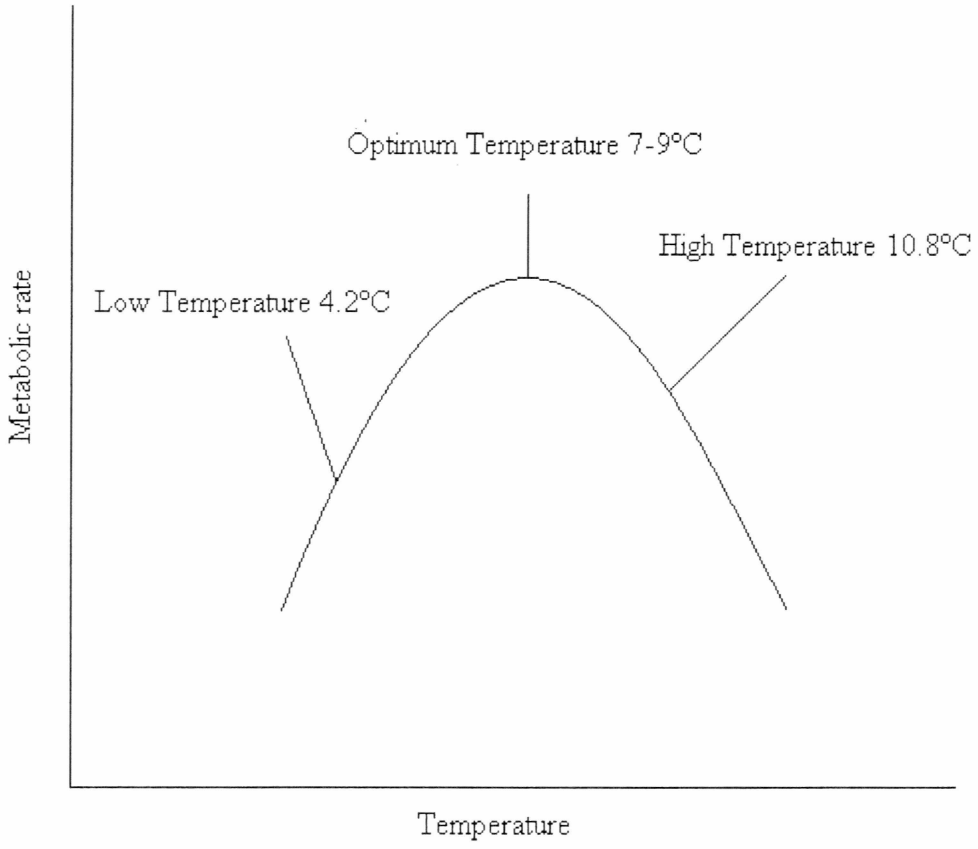

Figure 1.4. Suggested temperature optimum for Pacific cod.

Chapter 2. Effect of ambient temperature on recovery of Pacific cod (*Gadus macrocephalus*) following exhaustive exercise[1]

Post-exhaustive exercise recovery was examined in Pacific cod acclimated to either 2 or 7°C. Blood samples were collected prior to swimming, immediately after exhaustion and up to four hours of recovery and were analyzed for concentrations of cortisol as well as metabolites and ions. Concentrations of cortisol, glucose and lactate significantly increased post-swim as compared to samples collected pre-swim irrespective of temperature treatment. After four hours of recovery, samples remained elevated in cortisol, glucose and lactate and showed no indication of returning to pre-swim levels in either temperature group, indicating that four hours of recovery is not enough time to fully recover from the exhaustive exercise trials at either temperature tested.

Key words: Pacific cod; swimming performance; recovery; acclimation temperature; cortisol; metabolites; exhaustion.

[1]Hanna, S.K., Haukenes, A.H., Foy, R.J. & C.L. Buck. 2006. Effect of ambient temperature on recovery of Pacific cod (*Gadus macrocephalus*) following exhaustive exercise. Prepared for submission to the Journal of Fish Biology.

Introduction

Swimming performance is a critical aspect of the natural history of fish because it relates to migration, predator avoidance and prey capture (Beamish, 1978). Because swimming performance is such an important part of the natural history of fish, many studies have sought to examine performance in a variety of species (*Oncorhynchus kisutch* and *Oncorhynchus tshawytscha*, Davis *et al.*, 1963; *Oncorhynchus nerka*, Brett, 1964; *Gadus morhua*, Nelson *et al.*, 1996; *Oncorhynchus nerka* Farrell *et al.*, 1998; *Oncorhynchus kisutch* and *Oncorhynchus nerka*, Lee *et al.*, 2003; *Oncorhynchus clarki clarki*, MacNutt *et al.*, 2004; *Gadus morhua*, Martinez *et al.*, 2004; *Gadus morhua*, Herbert and Steffensen, 2005). There are a number of metrics of swimming performance that can be assessed using swim trials including sprint speed, rate of acceleration, and endurance swimming (see review by Wood, 1991). Critical swimming speed (U_{crit}) is an accepted evaluation of prolonged swimming performance (Beamish, 1978) and has been used widely to determine the effects of environmental change on the physiological condition of fish (Farrell *et al.*, 1998; Lee *et al.*, 2003; MacNutt *et al.*, 2004). In general, swim trials used to assess U_{crit} force a fish to swim in a flume against an increasing current for pre-set durations until it is exhausted and can no longer maintain its position in the flume. After exhaustion the fish is no longer capable of burst swimming (Milligan, 1996) and in the wild may be susceptible to predation and compromised in its ability to survive and acquire energy until it has sufficiently recovered. Therefore, the ability to

recover from exhaustion may limit subsequent swimming performance (Milligan, 1996) and impact survival.

Fish exposed to exhaustive exercise in the lab exhibit increased concentrations of plasma lactate in white muscle while glycogen reserves decrease and osmotic balance may be disturbed (Wood, 1991). Lactate from the tissue moves into the blood, although peak levels are not reached until 2 hours after exhaustion (Milligan, 1996). Recovery from exhaustive exercise is not well defined and there is debate as to what constituents must return to pre-swim values before the fish can be considered recovered (Kieffer, 2000). However, recovery from exhaustion generally involves the replenishment of tissue glycogen reserves, decrease of lactate concentrations and the normalization of tissue pH to pre-exercise values (Milligan and Wood, 1986; Wang *et al.*, 1994). Cortisol concentrations are also raised in fish swum to exhaustion and may inhibit replenishment of glycogen and removal of lactate (Milligan, 1996). To examine this concept, Pagnotta *et al.* (1994) blocked synthesis of cortisol in rainbow trout (*Oncorhynchus mykiss*). After exercise these fish recovered glycogen stores within 2 hours while control fish took 8 hours.

Time to recover following exhaustion varies substantially between studies even when examining the same species. Rainbow trout may take anywhere from 2 hours (Milligan *et al.*, 2000) to 24 hours (Milligan and Wood, 1986) after exhaustion to recover glycogen stores. Similarly, after 8 hours of recovery, rainbow trout had plasma lactate

concentrations that ranged from 2 (Kieffer *et al.*, 1994) to 6.4 fold (Milligan and Girard, 1993) that of pre-swim concentrations. Although recovery times differ, an exhausted fish will generally clear lactate to pre-swim levels within 12 hours (Kieffer *et al.*, 1994) and will recover glycogen reserves within 24 hours (Milligan and Wood, 1986).

Recent studies demonstrate that the rate of metabolic recovery can be influenced by temperature (Galloway and Kieffer, 2003); however, this relationship is not always clear. Wilkie *et al.* (1997) investigated the effect of acclimation temperature on metabolic recovery from exhaustive exercise in salmonids. They found that higher experimental temperatures (18-23°C) increased the rates of glycogen synthesis, lactate catabolism and restoration of pH balance following exhaustive exercise as compared to low temperature (12°C) acclimated fish. Kieffer *et al.* (1994) found that rainbow trout acclimated to higher temperatures (18°C) have greater concentrations of plasma lactate than low temperature (5°C) acclimated fish immediately post-swim. Although lactate catabolism was faster in high temperature acclimated fish, recovery time was similar in both groups due to different lactate concentrations post-swim. Kieffer *et al.* (1994) also found that glycogen recovery was independent of temperature and suggested that acclimation temperature does not significantly affect anaerobic capacity in rainbow trout. These contrasting views illustrate the need for further investigation into the effects of acclimation temperature on metabolic recovery post-exercise.

Pacific cod are distributed from the Yellow Sea to the Bering Strait and are distributed as far south as Los Angeles, CA. They are usually found on the continental shelf and upper slope and mainly live between 100-400 m depth (Cohen *et al.*, 1990). In the Gulf of Alaska, Pacific cod inhabit a region that is exposed to intra- and interannual variability in sea surface temperature as well as interdecadal variation in climate defined by the Pacific Decadal Oscillation (see review by Mueter, 2004). Interdecadal climate shifts have been associated with fluctuations in their distribution and abundance as well as that of other groundfish (Hare and Mantua, 2000).

Twenty-two Pacific cod (*Gadus macrocephalus*) divided between two temperature treatment groups (14 fish held at low temperature and 8 held at high temperature) and two recovery treatments (two hour and four hour) were used to evaluate physiological recovery following exhaustive exercise in Pacific cod. Blood samples taken both before and after swim trials and after 2 and 4 hours of recovery were analyzed for hematocrit and plasma concentrations of cortisol, lactate, glucose, total protein, Na^+, K^+ and Cl^- as indicators of condition and compared between treatments to determine recovery post-exhaustion at different temperatures. It was predicted that blood parameters would return to pre-swim values more quickly in the high temperature group than the low temperature group.

Materials and Methods

Adult Pacific cod, weighing 0.9-3.6 kg (44-68 cm total length) caught in waters surrounding Kodiak Island by pots (145 x 130 x 70 cm) in June of 2005 were transported back to the Kodiak Fisheries Research Center on Kodiak Island. Upon arrival in the wet lab, they were measured for total length on a measuring board (±1.0 cm) weighed (±0.01 kg). Maximum width and height were measured using a caliper just posterior to the pectoral fins (±0.1 mm). Each fish was marked using a unique number coded tag (T-bar tag FD-68B, Floy Tag®, Seattle, Washington) and weighed (±0.01 kg) prior to being placed into two 22 m^3 tanks supplied with sand filtered seawater drawn from Trident Basin at a depth of 25 m.

Swim trials began in November of 2005. Two weeks prior to swim trials, fish were randomly divided into low temperature (1-3°C) and high temperature (6-8°C) groups. The low temperature group consisted of six males and eight females with a mean mass of 1.89 ± 0.24 kg (range 0.60-3.46 kg). The high temperature group consisted of five males and three females with a mean mass of 1.92 ± 0.33 kg (range 1.18-3.62 kg). Fish were allowed to acclimate for at least two weeks prior to any further handling or testing. Fish were fed Pacific herring (*Clupea pallasii*) to satiation every three days.

After the two week acclimation period, each fish was captured from the tanks using a low abrasion dipnet and immersed in a 30 L solution of anesthetic (tricaine

methansulfonate; 100 ppm) for up to two minutes. Once anesthetized, each fish was placed on a V-board and 0.5 ml of blood was collected from the caudal vasculature using a 21 gauge needle and 3 ml syringe. Individual blood samples were placed into labeled vials coated with and anticoagulant, ammonium heparin, for the preparation of plasma samples. Hematocrit of each sample was determined in duplicate by drawing blood into micro-hematocrit capillary tubes (Fisherbrand®) following centrifugation at 12,000 RPM for 10 minutes (Unico® Micro-hematocrit). The remainder of the blood sample was refrigerated immediately (0-1°C) and was separated via centrifugation (2500 rpm for 15 minutes, Fisher Scientific accuSpin™ Micro R) ≤30 min of collection. Plasma was drawn off using a long-stemmed Pasteur pipette and placed into a labeled vial and stored at -50°C for later analysis. Fish were again measured for total length, standard length, width, height and weight as described above. Fish were then placed back into their respective tanks for an average of 27 days prior to swim trials.

Immediately preceding the swim trials, individual fish were fasted for either four days (high temperature group) or five days (low temperature group) to minimize the effect of digestion on metabolism. Fish were captured from tanks using a low abrasion dipnet and placed into a 370 L Brett-type swim flume maintained at the acclimation temperature of the fish. The swimming section of the flume was constructed of Lexan® and had a length of 1.87 m and a diameter of 20 cm. Prior to initiating the swim trial, fish were trained and acclimated to the swim flume to familiarize them with the swim protocol and to teach them to maintain position in the flume. This both familiarized the

fish with the swim protocol and trained them to maintain position in the flume while encouraging them to not rest at the back of the tunnel. Training as well as swim trials were performed as described in Hanna (2006). Measures of total length, width and height were used to calculate solid blocking effect of individual fish in the swim flume (Bell and Terhune, 1970). In brief, training consisted of exposing the fish to increases in water velocity for approximately 30 min. The fish was then allowed to acclimate to 0.5 BL s^{-1} for 36 h at which point 2 U_{crit} trials were performed with a 1 h rest period in between trials. During swim trials water velocity was increased 0.1 BL s^{-1} every 30 min until the fish was exhausted. Immediately after determination of the second U_{crit}, fish were removed from the swim flume and sampled for 0.5 ml of blood (as above). The fish was then placed back into its respective tank but separated from the other fish via netting. The fish was allowed to recover for either two or four hours. The tanks maintain a slow, circular current (<0.5 BL/s) thus allowing the fish to swim during the recovery period. After the recovery period, fish were removed and killed with a sharp blow to the head. A 1ml blood sample was obtained and fish were weighed and measured for standard morphometrics (as above).

Blood plasma was assayed for concentrations of glucose, lactate and protein using a SPECTRAmax® microplate spectrophotometer, SOFTmax® PRO software (Molecular Devices, Sunnyvale, California) and enzymatic assay kits (Glucose: Wako Pure Chemical Industries, Richmond, VA; Lactate: Trinity Biotech, St. Louis, MO; Protein: Pierce, Rockford, IL) according to manufacturers protocols adapted for use in

96-well microplates. Samples were assayed in triplicate to determine a mean optical density. Solutions of known concentrations of each constituent were also assayed on every plate to determine the linear relationship between optical density and concentration of the specific constituent. The mean intra-assay variation for the glucose, lactate and protein assays were 2.3%, 2.5% and 3.6%, respectively and the mean inter-assay variations were 4.2%, 9.9% and 9.4%, respectively.

Plasma sodium, chloride and potassium concentrations were determined using a Medica EasyElectrolytes ion meter (Medica Corporation, Bedford, MA). Samples were diluted with deionized water (11:5) to fit plasma concentrations into the working range of the instrument. All samples were assayed in duplicate. The mean intra-assay variation was 0.2%.

Concentrations of cortisol in plasma were determined using standard radioimmunoassay techniques originally described by Foster and Dunn (1974) and further modified by Redding *et al.* (1984). Samples were assayed in duplicate and the intra assay variation was <5%, inter assay variation was <10%. Briefly, 10 µl of sample was diluted with 200 µl glutamate buffer and heated in a water bath at 90-100°C for 15 minutes. Once cooled to room temperature, 0.04 µCi d ^{3}H cortisol in 500 µl phosphate buffer (pH 7.6) was added and whirl-mixed. A cortisol-specific antiserum diluted to bind 50% of the radio-labeled cortisol was then added and whirl-mixed. Samples were placed in an ice bath for five-minutes at which point ice-cold 2.5% dextran-coated charcoal was

added and the mixture was whirl-mixed. Samples were left in the ice bath for another five minutes before centrifugation. The supernatant was then decanted into individual vials containing scintillation cocktail. A standard curve was generated and used to calculate cortisol concentrations. Known concentrations of cortisol added to dextran-charcoal stripped Pacific cod plasma ran parallel with the standard curve.

Blood parameters were analyzed using two-way repeated measures analysis of variance with temperature and pre-swim, post-swim and post-recovery (time) as factors. Specific differences were determined using the Holm-Sidak method. SigmaStat 3.0 statistical software (Systat Software, Inc.) was used for all statistical analyses. All tests were performed at α=0.05. Data are presented as mean ± standard error of measure.

Results

The results of blood sample analyses are summarized in Figure 2.1. Plasma cortisol concentrations increased significantly from pre- to post-swim in both temperature treatments and recovery groups (2 hr: F=18.550, df=2, P<0.001; 4 hr: F=65.875, df=2, P<0.001). Concentrations of cortisol did not significantly differ between temperature treatments in either the two hour (F=0.0145, df=1, P=0.907) or four hour (F=0.00925, df=1, P=0.925) recovery groups. Cortisol concentrations were not significantly different from immediately post-swim levels after either two hours (low temperature: t=0.241,

P=0.813, high temperature: t=1.165, P=0.260) or four hours (low temperature: t=0.696, P=0.496, high temperature: t=0.755, P=0.460) in either temperature treatment.

Likewise, concentrations of plasma lactate increased significantly from pre- to post-swim in both the high temperature acclimated fish and the low temperature acclimated fish (2 hr: F=51.316, df=2, P<0.001; 4 hr: F=20.589, df=2, P<0.001) but were not significantly different between temperature treatments in either the two hour (F=1.517, df=1, P=0.249) or the four hour (F=1.632, df=1, P=0.233) recovery groups. Plasma lactate concentrations remained elevated in both temperature groups during recovery but were not significantly different from immediately post-swim samples after two hours (low temperature: t=0.491,P=0.629, high temperature: t=1.001, P=0.330) or four hours (low temperature: t=0.447, P=0.660, high temperature: t=0.119, P=0.907). Plasma protein concentrations were not significantly different between temperature groups in the two hour (F=0.454, df=1, P=0.517) or four hour (F=0.694, df=1, P=0.426) nor were they different between pre-swim, post-swim or post-recovery samples (2 hr: F=2.528, df=2, P=0.108; 4 hr: F=0.0634, df=2, P=0.939). Plasma glucose concentrations increased significantly from pre- to post-swim and further increased during recovery in both the two hour (F=6.960, df=2, P=0.006) and four hour (F=40.595, df=2, P<0.001) recovery groups. Concentrations of plasma glucose were consistently higher in the low temperature group than the high temperature group in both recovery groups. Glucose concentrations were significantly different between temperature groups in the two hour

recovery group (F=11.718, df=1, P=0.008) but not in the four hour (F=3.215, df=2, P=0.107) recovery group.

A statistically significant interaction existed between temperature and time for plasma Na^+ concentrations in the two hour recovery group (F=4.690, df=1, P=0.023) but increased in both temperature groups in the four hour recovery group from pre- to post-swim and during recovery (F=2.368, df=2, P<0.001). Plasma K^+ concentrations increased from pre- to post-swim in both recovery and temperature groups (2 hr: F=23.391, df=2, P<0.001; 4 hr: F=65.554, df=2, P<0.001). Plasma Cl^- concentrations were consistently higher in the low temperature group but only significantly higher in the two hour recovery group (F=160.988, df=1, P<0.001). A statistically significant interaction existed between temperature and time for plasma Cl^- concentrations in the four hour recovery group (F=5.890, df=2, P=0.011). Cl^-concentrations significantly increased from pre- to post-swim and during recovery in the two hour recovery group (F=4.670, df=2, P=0.023).

A statistically significant interaction existed between temperature and time for hematocrits in the two hour recovery group (F=4.838, df=2, P=0.022) and did not significantly differ between temperature groups in the four hour recovery group (F=0.0452, df=1, P=0.836). Additionally, hematocrit values significantly decreased from pre-swim to recovery in the four hour recovery group (F=4.871, df=2, P=0.020).

Discussion

The results of this study corroborate earlier work in which Pacific cod acclimated and swum at two different temperatures did not differ in their swimming performance or rates of oxygen consumption (Hanna, 2006). The current study demonstrates that recovery of Pacific cod from exhaustive exercise is incomplete after four hours as evidenced by elevated concentrations of plasma cortisol, glucose, lactate and ions and is not dependent on temperature. These results contrast with other studies in which fish held at higher temperatures recover faster during a four hour recovery period than those held at lower temperatures (Wilkie *et al.*, 1997; Galloway and Kieffer, 2003).

Similar to this study, rainbow trout exhibit increased concentrations of plasma glucose post-exercise and during recovery and remain elevated four hours after exhaustion (Milligan and Wood, 1986; Wood, 1990). Also, concentrations of glucose found in this study are similar to those found for Atlantic cod in which pre-swim plasma glucose was between 4 and 7 mmol/L and after four hours of recovery was between 8 and 10 mmol/L (Nelson *et al.*, 1996). Glucose concentrations increase in stressed (Wendelaar Bonga, 1997) and exercised fish due to glycogenolysis and gluconeogenesis (Moyes and West, 1995) associated with increased energy demands and may also increase due to increases in plasma cortisol. Cortisol aids in the mobilization of energy stores and has been shown to stimulate release and synthesis of glucose thus resulting in increased concentrations of plasma glucose (Gamperl *et al.*, 1994; Mommsen *et al.*, 1999).

However, the differences in glucose concentrations between temperature groups found in this study are not consistent with a previous study on Pacific cod (Hanna, 2006) and are only significant within the two hour recovery group and not the four hour recovery group. This may be indicative of higher rates of glycogenolysis/gluconeogenesis in the low temperature acclimated fish or a reduced usage of glucose in these fish compared to high temperature acclimated fish. Similar differences in plasma Cl^- concentrations between temperature groups combined with differences in plasma glucose concentrations suggest a stress associated with the low temperature group.

Cortisol, lactate, glucose and ions did not return to resting levels after four hours of recovery in this study. Cortisol plays a key role in recovery by slowing the process through influences on lactate metabolism and glycogen resynthesis (Milligan, 1996). When cortisol synthesis is blocked, rates of lactate clearance and glycogen resynthesis are increased (Pagnotta *et al.*, 1994). Cortisol levels may remain elevated for several hours (Milligan, 1996) or days to weeks (Haukenes and Buck, 2006) following exposure to a stressor. Milligan *et al.* (2000) found that cortisol does not increase in rainbow trout that are allowed to swim at low velocity after exhaustive exercise and thus lactate is cleared within two hours of recovery. This suggests that the fish in this study, although swimming during recovery, may not have been swimming fast enough to limit cortisol accumulation and thus recovery may have been delayed.

Plasma Cl^- and Na^+ concentrations were consistently higher in the low temperature fish than that of high temperature fish throughout the experiment. Increased plasma ion concentrations are indicative of an osmotic stress (Redding and Schreck, 1983). Fish in the low temperature group were not able to ionoregulate as well as their high temperature counterparts due to the stress associated with the lower temperatures. Redding and Schreck (1983) also indicate decreases in hematocrit associated with stress, yet, this was not seen in either temperature or recovery groups in this study. The increases in plasma lactate and glucose in association with exercise has been seen in other studies (Milligan and Wood, 1986; Wood *et al.*, 1990), and the lack of any change in plasma protein is also comparable to other studies (Wang *et al.*, 1994).

Although temperature seemed to have little impact on the recovery process in this study, the differences seen in other studies occurred over wider temperature ranges than those examined in this experiment and included three temperature groups. Wilkie *et al.* (1997) acclimated Atlantic salmon to 12, 18 and 23°C and Galloway and Kieffer (2003) examined Atlantic salmon at 6, 12 and 18°C. Additionally, Galloway and Kieffer (2003) exposed fish to an acute temperature change as opposed to maintaining fish at their acclimation temperature. It is possible that the temperatures examined in this experiment were on either side of the optimum temperature for Pacific cod and therefore differences in recovery may still exist in Pacific cod acclimated to different temperatures.

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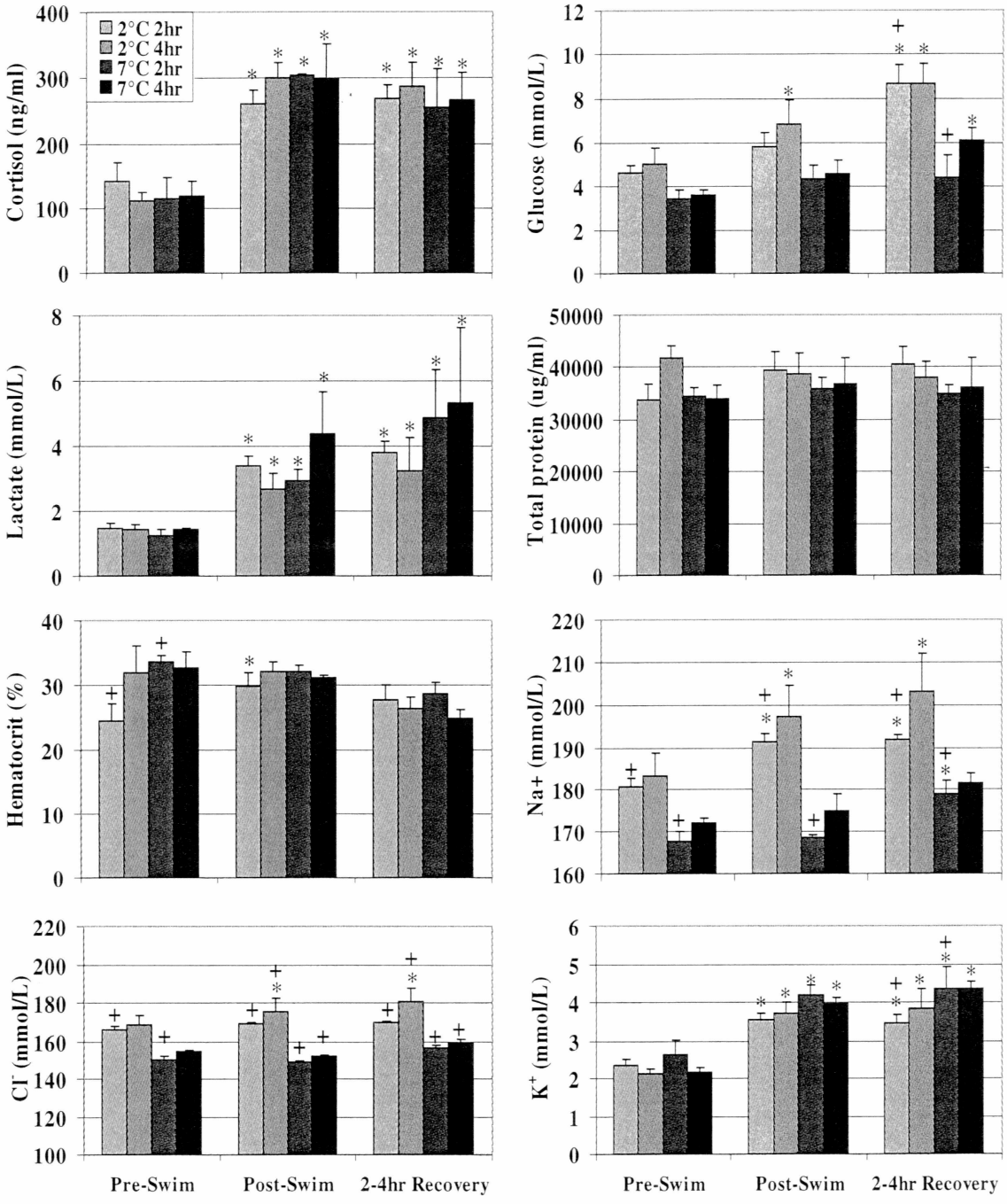
Figure 2.1. Blood constituents ± *SE* for Pacific cod acclimated to 2 and 7°C. Blood samples were taken prior to swimming, immediately after exhaustion and at either two or four hours of recovery post-exhaustion. Significant differences ($P<0.05$) from pre-swim values are indicated by "*" and differences between temperature groups are indicated by "+". Statistically significant interactions existed between temperature and time for plasma Na^+ concentrations ($F=4.690$, $df=1$, $P=0.023$) and hematocrits ($F=4.838$, $df=2$, $P=0.022$) in the two hour recovery group and for plasma Cl^- concentrations in the four hour recovery group ($F=5.890$, $df=2$, $P=0.011$).


2°C 2hr
2°C 4hr
7°C 2hr
7°C 4hr
Cortisol (ng/ml)
Glucose (mmol/L)
Lactate (mmol/L)
Total protein (ug/ml)
Hematocrit (%)
Na+ (mmol/L)
Cl- (mmol/L)
K+ (mmol/L)
Pre-Swim
Post-Swim
2-4hr Recovery
Pre-Swim
Post-Swim
2-4hr Recovery

Conclusions

Changes in abundance of marine organisms in the Pacific Ocean associated with shifts in climate (Hollowed and Wooster, 1992; Anderson and Piatt, 1999; Peterson and Schwing, 2003) as well as indications of a recent shift (Schwing and Moore, 2000; Peterson and Schwing, 2003) have sparked interest in the effects of environmental conditions on economically important species. Pacific cod (*Gadus macrocephalus*), which made up 12.5% of the groundfish catch in Alaska in 2004 (Hiatt *et al.*, 2005), were used to examine how temperature influences physiology. Two experiments were conducted to determine critical swimming speed (U_{crit}), rates of oxygen consumption and recovery post exhaustion of adult cod acclimated to different temperatures. In addition, hematocrit and plasma concentrations of cortisol, glucose, lactate, total protein, sodium, chloride and potassium from resting and exhausted fish were measured to assess the impact of swim trials on fish condition. Based on similar studies with other species it was believed that Pacific cod acclimated to higher temperatures would demonstrate greater U_{crit}, higher oxygen consumption rates and more rapid time to recovery than those acclimated to lower temperatures.

Data from experiment one illustrate that Pacific cod acclimated and exercised at 4°C consume similar amounts of oxygen and perform just as well as those acclimated and exercised at 11°C. Blood samples taken before and after swims suggest slight osmotic differences between temperature groups but few differences in metabolites. The impact of

acclimation temperature on metabolic rate of Pacific cod was not significant and the Q_{10} was below 2, suggesting that these fish acclimate to maintain metabolic rate when exposed to fluctuating temperatures as those seen in interdecadal climate changes. These results suggest that the acclimation temperatures chosen have little effect on metabolic rate and swimming performance. Results from experiment two demonstrate that Pacific cod acclimated to either 2 or 7°C are not fully recovered from exhaustive exercise after four hours post-exhaustion as evidenced by elevated concentrations of plasma cortisol, glucose, lactate and ions. Similarly recovery rates of cortisol, metabolites and ions did not differ between the temperature groups, illustrating that recovery rate is not dependent on temperature.

The reasons for changes in Pacific cod abundance as well as the abundance of other fish and shellfish with shifting regimes is unknown (Francis *et al.*, 1998, McGowan *et al.*, 1998). However, based on the results from these two experiments, it seems unlikely that physiological constraints of adult Pacific cod contribute to changes in their relative abundance. Either physiological constraints on earlier life stages are affecting Pacific cod abundance or ecological constraints may be driving Pacific cod to move based on prey and/or predator abundance.

Data from this study may be used in bioenergetics models to better predict energy needs of Pacific cod in different environments. Future work should examine ecological effects of regime shifts on Pacific cod abundance as well as temperature impacts on the

physiology of the prey and predators of Pacific cod. Environmental temperatures may have greater impacts on other marine organisms that Pacific cod rely on, thus indirectly affecting the abundance of these fish.

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